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## MOLECULAR VERIFICATION OF *CRYPTOPTS HORTENSIS* (SCOLOPENDROMORPHA: CRYPTOPIDAE) IN THE NEARCTIC REGION<sup>1</sup>

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**ABSTRACT:** *Cryptops hortensis* is a successful invasive species probably of Palearctic origin. However, recent molecular studies in Europe identified multiple similar species previously misidentified as *C. hortensis*. In the Nearctic Region, *C. hortensis* is presumably an exotic species with well-established populations in the northeastern USA. After molecular and morphological identification, *C. hortensis* from Ohio, USA, were identified as the same species in Europe. The molecular data supported the hypothesis that *C. hortensis* was introduced from Europe.

**KEY WORDS:** Centipede, Exotic Species, Molecular Biology, Myriapod, *Cryptops leucopodus*

*Cryptops hortensis* (Donovan) (Scolopendromorpha: Cryptopidae) is a Holarctic centipede that was possibly introduced into North America from Europe (Crabill, 1960). *Cryptops hortensis* is a rarely recognized but successful invasive species found across Europe, parts of Asia, and North America, often in synanthropic habitats (Shelley, 2002; Nefediev, 2016). Older scattered collection reports indicate possible independent introductions in North America, and Shelley (2002) supported this by presenting a disjunct distribution of *C. hortensis* across North America. The identifications by Shelley (2002) were based solely on morphologic characters. *Cryptops hortensis* in the Americas might be a species complex or at least several morphologically similar species that were mistakenly placed in synonymy as was demonstrated in Europe by molecular taxonomic studies by Wesener et al. (2016). An alternative hypothesis is that *C. hortensis* in the Americas is a unique species distinct from that in Europe. As a component of a larger scale molecular study of North American centipedes, I conducted a molecular barcoding experiment to determine if specimens identified as *C. hortensis* in Ohio, USA, represent *C. hortensis* s.s., an unknown native species, or another Palearctic species.

### METHODS

*Cryptops hortensis* were collected from three locations (Rosewood City Park 39.604 N, 84.178 W, Montgomery Co.; a residential garden, 39.598 N, -84.179 W, Montgomery Co.; and Wright-Patterson Air Force Base, 39.781 N, -84.089 W, Greene Co.) in Ohio, USA, from 29-31 March, 2016. For comparison, two *Cryptops leucopodus* (Rafinesque) were collected in Georgia (Marietta 33.901 N, -84.444 W, Cobb Co.; and Atlanta 33.840, -84.284, DeKalb Co.) on 9-10 April, 2016.

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Two legs were removed from each of 9 *C. hortensis* and 2 *C. leucopodus* using a sterile scalpel and macerated with a polypropylene pestle in sterile microcentrifuge tubes. Total nucleic acids were extracted with a Maxwell® 16 Viral Total Nucleic Acid Purification Kit (Promega, Madison, Wisconsin) following the manufacturer's protocol. A fragment of the cytochrome oxidase I (CO-I) gene was amplified by PCR using primers LCO1490 and HCO2198 following the protocols by Folmer et al. (1994). These primers were also used by Wesener et al. (2016) when studying morphologically similar species of *Cryptops* spp. in Europe. A water negative control and a positive control consisting of a genomic extract from *Thereuonema tuberculata* (Chilopoda: Scutigero-morpha) were used. PCR products were detected using ethidium bromide in a 2% gel electrophoresis with ultraviolet light. PCR products were then cleaned up using a QIAquick PCR Purification Kit (Qiagen, Valencia, California). Sequencing reactions were done with PCR primers using a BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, California), and excess dye was removed by ethanol precipitation. Sequences were determined using an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, California), by DNA Analysis, LLC (Cincinnati, Ohio). Primer sequences were removed and the sequences were aligned and assembled with ClustalW (Kyoto University Bioinformatics Center, Japan), and compared to sequences in GenBank using the BLAST program (NCBI, Bethesda, Maryland). Voucher specimens in ethanol were deposited in the Museum of Comparative Zoology, Harvard University. Unique CO-I sequences were submitted to GenBank as *Cryptops hortensis* (GenBank#s: KY741553, KY741554, KY741555, KY741556, KY741557, KY741558, KY741559, KY741560, KY741561, KY741562, and KY741563) and *Cryptops leucopodus* (GenBank #s: KY741562 and KY741563).

## RESULTS AND DISCUSSION

All nine centipedes morphologically identified as *C. hortensis* had 674 bp CO-I sequences 99-100% identical to *C. hortensis* sequences on Genbank (KM491678, KM491565, KU497162) from Germany. Both *Cryptops leucopodus* were closest (85%) to an unidentified *Cryptops* sp. ZFMK-TIS-9755 (GenBank# KM491620) from Austria and 80% similar to the *C. hortensis* sequences.

Both morphological and molecular characters support the status of *C. hortensis* as an introduced European species in Ohio. This supports the status of *C. hortensis* as presented by Shelley (2002) and was not completely expected, because recent molecular studies by Wesener et al. (2016) in Europe demonstrated that several species were misidentified as *C. hortensis*. In addition, there was no reason to assume that the North American populations were genetically similar to those from Europe. The CO-I of *Cryptops leucopodus* was divergent compared to *C. hortensis*, as would be expected when comparing a Nearctic species to a distantly related Palearctic species.

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